

Chromatin Immunoprecipitation

A) Prepare a yeast culture (see the *Galactose Induction Protocol* for details).

- 1) Start a small culture (e.g. 2 ml) in YEPD or selective media from a single colony.
- 2) Spin down the cells and keep the pellet; resuspend cells in YEP -lactate. Repeat the spin and resuspension in YEP -lactate (4 mls). Incubate at 30° for several hours or more.
- 3) Inoculate a large culture and allow to grow overnight at 30°. Adjust inoculum size so that the culture will be between 3×10^6 and 1×10^7 cells/ml the next day.

B) Chromatin Crosslinking with Formaldehyde

- 1) When the culture is between 3×10^6 and 1×10^7 cells/ml dilute a sample 1:5 using YEP-lactate and then take the OD₆₀₀ (see misc. notes). Harvest a 45 ml sample in a 50 ml conical tubes. Crosslink the cells by transferring the culture to a 50 ml conical tube containing 1.2 mls formaldehyde (37% stock solution or 1% final concentration). Incubate the sample on a platform-rocker for 10 min at room temperature.
- 2) Dilute a small aliquot of cells by 10^{-4} and plate 100 ul (before induction and at approx. 4 hr).
- 3) Quench the crosslinking by adding 2.5 ml of 2.5 M glycine and incubate on a platform-rocker for 5 minutes at room temperature.
- 4) Centrifuge for 5 min, discard the supernatant and resuspend the cells in 1 ml of ice cold TBS in a 1.5 ml tube. Wash two additional times with 1 ml of TBS. Remove as much supernatant as possible. After the final spin, remove the supernatant and freeze the pellet at -70°.

C) Cell Lysis and Sonication

- 1) Resuspend the cell pellet in 0.55 ml of cold lysis buffer. Transfer the cell lysate to a 1.5 ml microcentrifuge tube containing 0.5 ml of glass beads (425-600 μ m diameter, acid washed) on ice. Lyse the cells on a multi-tube vortexer for 30 to 60 min at 4° until 90% lysis is achieved.
- 2) Make a very small hole in the bottom of the tubes with a red hot syringe needle, heated with a Bunsen burner. Place the tubes on top of 15 ml conical tubes on ice and centrifuge the tubes to separate the whole cell lysate from the glass beads.
- 3) Resuspend the pellet in the supernatant and transfer the lysate to 2 ml tubes. Sonicate the samples on ice until the DNA has an average size of 500 bp.

- 4) Centrifuge the lysate for 10 min at 4°, transfer the supernatant to a new tube and repeat the spin. Transfer the supernatant (“Pre-IP”) to a new tube on ice and freeze at –70°.

D) Chromatin Immunoprecipitation

- 1) For the non-immunoprecipitated (“Non-IP” or “Input”) samples, aliquot 50 µl of lysate to a new tube. Keep on ice until the immunoprecipitated (IP) sample is ready for reversing the crosslinks.
- 2) For the IP sample, add 350 µl of lysate to a chilled tube containing the antibody. Freeze the remaining lysate and save for use as the pre-IP control on a Western blot. Incubate the IP tubes at 4° with gentle agitation for 1 hr.
- 4) Gently spin protein-G or -A agarose beads down at 3000 rpm for 1 minute in a 2 ml tube and remove the supernatant. Wash the beads twice with 1 ml of lysis buffer. Resuspend the beads in an equal volume of lysis buffer. Add 30 µl of the protein-G or -A agarose beads to each IP sample. Incubate the tubes at 4 °C with slow rocking or rotating for 1 hr.
- 7) Pellet the beads for 1 min at 3000 rpm. Remove the supernatant and save at –70°. Use a 5 ul aliquot for the “cleared-lysate” or “post-IP” sample if running a Western blot to check the efficiency of the antibody binding to the protein. Wash the beads for 5 min at room temperature, centrifuging at 3000 rpm for 1 min:
 - 2x lysis buffer
 - 1x high salt lysis buffer
 - 1x wash buffer
 - 1x TE
- 9) Elute the precipitate by adding 100 µl of elution buffer and incubating at 65° for 15 min.
- 10) Pellet the beads for 1 min at 13000 rpm and save the eluate in a fresh tube. Add 150 µl of TE/0.67% SDS solution to the tubes containing the beads and incubate at 65° for 10 min. Pellet the beads for 1 min and combine with the eluates above. Add 200 µl of TE/1% SDS solution to the Non-IP samples and incubate both the IP and Non-IP samples at 65° for 6 hr to reverse the crosslinks.
- 14) Add 250 µl TE containing proteinase K (0.4 mg/ml) and glycogen (0.1 mg/ml). Incubate the samples at least 2 hr at 37°.

E) DNA Precipitation and PCR

- 1) Add 55 μ l of 4M LiCl to the samples and extract with 500 μ l of phenol in new tubes. Centrifuge and save the supernatant. Precipitate the DNA with 1 ml of ethanol for 15 min at room temperature and centrifugation. Wash the pellet with 80% ethanol. Centrifuge for 10 min and discard the supernatant. Allow the pellets to dry and dissolve the DNA in TE.
- 2) The DNA can be analyzed using conventional or real time PCR. For conventional PCR, a typical program is: 1 min 94°, 26 cycles of (15 sec 94°, 20 sec 55°, 20 sec 72°) and 5 min at 72°. PCR products are chosen to be about 300 bp long and can be visualized on a 2% agarose gel. Adjust the conditions to obtain signals in the linear range of a calibration curve prepared with dilutions of the Non-IP 0 hr sample. If using real time PCR, the products should be 100 to 300 bp in length and the 0 hr Non-IP sample can be used for the standardization curve. A hot-start *Taq* polymerase is recommended to minimize competing PCR products. For controls, Non-IP and IP samples from an independent locus should be amplified and quantitated. A sample not treated with formaldehyde provides a control to determine whether protein binding occurred after lysis.

F) The size range of the sonicated DNA can be checked by reversing the crosslinks of the Non-IP samples, treating with RNase and running the DNA on a 0.8% agarose gel.

Comments

Harvesting cells. There is a noticeable increase in the size of the cell pellet in time courses lasting 4 hr or longer. To compensate for this, smaller samples are taken at later time points and diluted with YEP-lactate to bring the volume up to 45 ml, based on OD₆₀₀ measurements.

Optimizing ChIP. There are several variables in the ChIP protocol that may be optimized including the degree of crosslinking, cell lysis and sonication of DNA. The degree of crosslinking can be varied by changing the incubation time with formaldehyde. The conditions described in this protocol work well for many proteins, but they may need to be altered depending on the protein. Cell lysis should also be optimized since it is dependent on the vortexer. Intact cells can be counted under a microscope using a hemacytometer. Sonication conditions also should be adjusted such that the DNA size average is about 500 bp. The quality and effectiveness of the antibody should also be checked by examining the Non-IP and Post-IP samples on Western blots. The optimal antibody dosage should also be determined.

Normalization of PCR signals. There are a number of different ways to normalize ChIP signals. We describe two methods here. The first method is to normalize the IP time point signals to the input signal at 0 hr for the locus of interest (e.g. the *MAT* site) and the independent control locus. If using real time PCR, this is conveniently accomplished by using dilutions of the 0 hr Non-IP sample for the standardization curve. The ratios of the *MAT* IP to control IP signal for each time point are then normalized to the 0 hr time point. The second method normalizes the IP signals to the input signal at 0 hr for both the *MAT* and control loci as above. The ratio of the *MAT* IP signal to the control Non-IP signal can then be calculated for each time point. This method is useful if the signals from the 0 hr IP and/or control IPs are very low or if the *MAT* and control IP signals are not completely independent.

Solutions:

TBS (Tris buffered saline): 20 mM Tris pH 7.6, 150 mM NaCl

Lysis Buffer: 50 mM HEPES pH 7.5, 1 mM EDTA, 140 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mg/ml Bacitracin, 1 mM benzamidine, 1 µg/ml leupeptin, 1 µg/ml pepstatin A, 1 mM PMSF.

High Salt Lysis Buffer: same as Lysis buffer except 0.5 M NaCl,

Wash Buffer: 10 mM Tris pH 7.5, 1 mM EDTA, 0.25 M LiCl, 0.5% IGEPAL CA-630, 0.5% sodium deoxycholate

TE: 10 mM Tris pH 7.5, 1 mM EDTA

Elution Buffer: 50 mM Tris pH 7.5, 1 mM EDTA, 1% SDS

TE/0.67%SDS: 10 mM Tris pH 7.5, 1 mM EDTA, 0.67% SDS

TE/1%SDS: 10 mM Tris pH 7.5, 1 mM EDTA, 1% SDS

This procedure is based on the method of Strahl-Bolsinger et al. (1997) and Evans et al (2000). We are indebted to E. Evans, T. Goldfarb and E. Alani for helping us with this procedure.

Strahl-Bolsinger, S., et al., *SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast*. Genes Dev, 1997. 11(1): p. 83-93.

Evans, E., et al., *The Saccharomyces cerevisiae Msh2 mismatch repair protein localizes to recombination intermediates in vivo*. Mol. Cell., 2000. 5(5): p. 789-799.

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